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INTERNATIONAL BULLETIN
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BACTERIOLOGICAL NOMENCLATURE
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Volume 10 July 15, 1960 No. 3

FLAGELLATION IN SIX SPECIES OF SPIRILLUM - A CORRECTION[1]

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Williams and Rittenberg (1957) described six species of the genus Spirillum Ehrenberg as having a single flagellum at each pole in flagellar-stain preparations. Five of the species—Spirillum lunatum, S. curvatum, S. atlanticum, S. beijerinckii and S. anulus—were new species; the sixth species was the well-known S. volutans. In the discussion of flagellation in species of Spirillum Williams and Rittenberg mention the fact that when Leifson's (1951) method of flagellar staining was used that the so-called 'single flagellated' species often showed multiple flagella. Inasmuch as variable results were obtained by routine flagellar stain methods, these authors recommended a survey of flagellation in species of Spirillum by means of the electron microscope to resolve this difficulty.

Such electron microscope photographs have been made and it was found that all six species, mentioned above, have multiple flagella. In some instances, even in electron microscope photographs, the flagella are tightly agglutinated to each other, and in flagellar-stain preparations would probably appear as a single flagellum. Such a fascicle of flagella is shown in S. atlanticum in figure 1. In other preparations, the flagella are separated as is shown in figure 2 of S. volutans.

Due to the fact that the six species of Spirillum, described by Williams and Rittenberg (1957) as having a single

[1]This investigation was supported by a research fellowship (EF-9726) from the National Institute of Allergy and Infectious Diseases. U.S. Public Health Service.

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Figure 1. Electron microscope photograph of S. atlanticum showing multiple flagella which are agglutinated together. Magnification 48, 000 X.

Figure 2. Electron microscope photograph of S. volutans showing multiple flagella. Magnification 33, 500 X.

flagellum at each pole, have been shown to possess multiple flagella, the description of these species should be emended to read: "motile by tufts of bipolar flagella".

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STATUS OF SYNONYMY AND PLANT PATHOGENICITY OF PSEUDOMONAS MARGINALIS AND P. AERUGINOSA[1]

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A recent statement that Pseudomonas aeruginosa is a plant pathogen as well as an animal pathogen (35) and reports to be discussed below that the plant pathogen P. marginalis is identical with P. aeruginosa make it desirable to examine the question of the synonymy of these names and the role of P. aeruginosa as a plant pathogen.

Literature Survey

In 1918 N. A. Brown described P. marginalis as the cause of a marginal leaf discoloration of lettuce. The new, fluorescent species was reported to have a maximum growth temperature at 38°C; no mention was made of the formation of pyocyanin (4). In 1924 Mehta and Berridge reported one subculture of P. marginalis, originally received by S. G. Paine from N.A. Brown, as identical with P. aeruginosa. No report was made on pyocyanin production or on the maximum growth temperature. The strain of P. marginalis used differed in several respects from Brown's original description, viz., it produced a greenish discoloration on steamed potato and failed to produce acid from sucrose. A

[1]The following synonymy is used (2,14): Pseudomonas aeruginosa (Schroeter) Migula (Bacterium aeruginosum Schroeter, Bacillus pyocyaneus Gessard, Pseudomonas pyocyanea Migula); Pseudomonas marginalis (Brown) Stevens (Bacterium marginale Brown, Phytomonas marginalis (Brown) Bergey et al., Phytomonas intybi Swingle); Pseudomonas polycolor Clara (Phytomonas polycolor Clara, Bacterium polycolor (Clara) Burgwitz); Pseudomonas aptata (Brown and Jamieson) Stevens (Bacterium aptatum Brown and Jamieson, Phytomonas aptata (Brown and Jamieson) Bergey et al).

culture of P. aeruginosa, apparently not obtained from diseased plants, was pathogenic to lettuce upon inoculation (26). At the same time Paine and Branfoot stated P. aptata isolated from diseased lettuce was the same as P. aeruginosa. The lettuce isolate showed a maximum growth temperature below 37°C, and no test for pyocyanin was reported or was a comparison made with a culture of P. aeruginosa (30). St. John-Brooks et al. studied 2 cultures of P. marginalis, one labelled E. F. Smith was apyocyanogenic, the other labelled N. A. Brown was pyocyanogenic. Antiserum of P. marginalis E. F. S. did not agglutinate P. marginalis N. A. B. or 11 cultures of P. aeruginosa. P. aeruginosa antiserum did not agglutinate P. marginalis E. F. S. or any other phytopathogenic pseudomonads studied (38). From diseased tobacco Clara described P. polycolor which had a maximum growth temperature of 39°C and failed to produce pyocyanin. A culture of P. aeruginosa, apparently from a non-plant source, failed to produce infection upon inoculation of 17 different plant hosts (7, 8). Lacey reported a number of fluorescent isolates from diseased lettuce resembled P. marginalis; all showed no growth or feeble growth at 37°C with no mention made of pyocyanin formation (23). Baldacci and Ciferri reported a strain of P. aeruginosa, of human origin, caused a type of tomato infection upon inoculation (1). Desai obtained from rotting sugarcane an organism reported to resemble P. marginalis and 2 other lettuce pathogens. The sugarcane organism was believed to belong to the P. aeruginosa group. No mention was made of pyocyanin formation, the growth temperature range of the isolate, nor was it compared with a culture of P. aeruginosa. Upon inoculation it caused a weak infection of sugarcane and failed to produce disease on 7 other hosts (11). Verona and Pasinetti obtained slight infection of frost-injured lettuce upon inoculation with a culture of P. aeruginosa (40). Elrod and Braun (15) obtained plant infections with a number but not all 15 strains of P. aeruginosa tested, apparently of human, animal, water or other non-plant origin, when an excessive inoculum was used on weakened host tissue. They reported a laboratory strain of P. polycolor produced pyocyanin, and speculated, without a culture study, that P. marginalis might be synonymous with P. aeruginosa (15). Reid et al. studied 27 strains of P. aeruginosa and reported all showed good growth at 37°C, although 10 of the strains

did not produce pyocyanin (31). Verona speculated that some saprophytes which produce proteolytic enzymes are able to cause a rot in plants which contain a large amount of nitrogen. He reported that when grown on peptonized meat agar P. aeruginosa could cause a rot of cabbage (39). Dowson stated that pyocyanogenic cultures labelled P. marginalis were considered to be P. aeruginosa while fluorescent, apocyanogenic cultures were P. marginalis (13). Friedman identified a number of fluorescent pseudomonads from diseased lettuce, escarole, and witloof chicory as P. marginalis. None of the isolates produced pyocyanin and all had no growth or weak growth at 38°C (16, 17). Haynes characterized P. aeruginosa as producing pyocyanin. Apycyanogenic strains could be identified by their ability to grow at 42°C. A laboratory culture of P. marginalis from England and P. polycolor were found to be pyocyanogenic (22). Friedman reported antiserum of P. marginalis (strain 249) showed no agglutination of 2 strains of P. marginalis (strains 136, CIBP), 5 strains of P. fluorescens or 11 named fluorescent, phytopathogenic pseudomonads, and a low titer reaction with P. aeruginosa (A.T.C.C. 10145) which indicated lack of serological identity (18). In a study of P. marginalis isolated from diseased lettuce and cabbage, Burkholder stated the ability of P. aeruginosa to produce pyocyanin and to grow at a high temperature range were not possessed by P. marginalis (5). Okabe and Goto reported 2 strains of P. aeruginosa, apparently from non-plant sources, caused a weak infection of carrot and potato but none on 15 other plant hosts. The 2 strains produced pyocyanin but other fluorescent pseudomonads, as P. marginalis, did not. P. marginalis was reported to be distinct pathologically from P. aeruginosa (28). Volcani reported a green fluorescent organism isolated with other organisms from diseased banana plants caused lesions on 6 plant hosts but not on bananas. The pseudomonad had optimum growth at 30°, grew at 43° and was fatal to mice. It was believed to be either P. polycolor or P. aeruginosa (41). Stapp reported only 1 of a number of strains of P. aeruginosa infected potato tubers; the strain was of human origin(37).

Discussion

P. marginalis as described originally (4) and similar

strains subsequently isolated from diseased plants (5, 16, 17, 21, 23, 28) have not been reported to grow at 41°C or to produce pyocyanin. On the other hand, P. aeruginosa has been characterized by its ability to grow at 41°C and to produce pyocyanin typically (2, 8, 22, 24, 31). In addition, agglutination tests indicate that P. marginalis and P. aeruginosa are serologically different (18, 38). A number of workers have reported P. marginalis to be a distinct species (2, 5, 13, 14, 16, 25).

Bacteria causing soft rot of plants produce pectolytic enzymes, and it has been shown that P. marginalis forms pectolytic enzymes in culture and ferments pectic substances (6, 19, 21, 29, 36). P. aeruginosa has not been reported to do so (36). Strains of P. marginalis which have lost their pathogenicity during the course of long laboratory cultivation or as a result of mutation resulting from ultraviolet light irradiation have been found to have lost their power to form pectolytic enzymes or to ferment pectic substances (19, 32). Such strains can be differentiated from P. aeruginosa on the basis of maximum growth temperature or pyocyanin characteristics but are distinguished with difficulty from P. fluorescens (Friedman, unpub. data).

P. marginalis has been reported repeatedly to be a virulent plant pathogen (3, 4, 5, 9, 10, 12, 16, 17, 20, 21, 23, 27, 28, 33, 34). In contradistinction, P. aeruginosa has been reported isolated from naturally diseased plants in only a relatively few instances (11, 30, 41), and in no case have the isolates been described in sufficient detail to make possible a definitive determination of the species of Pseudomonas involved or have Koch's postulates of pathogenicity been fulfilled. Most strains of P. aeruginosa, from human, animal, soil or other non-plant sources, have failed to produce infections in plants upon inoculation (9, 23, 37), but in a number of instances weak plant infections have resulted following inoculation with other strains (1, 15, 28, 40). At present it would appear that until such time as P. aeruginosa is definitely isolated from naturally diseased plants and Koch's postulates of pathogenicity fulfilled, the role of P. aeruginosa as a plant pathogen is doubtful. It should be noted that the status of P. polycolor, said to be synonymous with P. aeruginosa (15), as a species is doubtful. As far as can be determined from the literature, P. polycolor has not been reported isolated from diseased tobacco or any other source since 1930 when it was first described.

Conclusion

Pseudomonas marginalis is the correct name of a distinct species, and is not a synonym of P. aeruginosa. P. marginalis can be differentiated from the latter species by the following characteristics. It does not form pyocyanin, shows no growth or feeble growth at 37° to 39°C, produces pectolytic enzymes in cultures, ferments pectic substances, and is a virulent plant pathogen causing natural infections on a number of plant hosts. The role of P. aeruginosa as a plant pathogen is considered doubtful at present.

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PROPOSED PHYLOGENY IN THE FAMILY BACILLACEAE

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The family Bacillaceae enjoys the distinction among true bacteria of having a readily identifiable, taxonomically acceptable, characteristic in the endospores which serve to differentiate it from other families of the Eubacteriales. The large number of distinct and well characterized species allow comparison of species characteristics within the family. It provides a logical starting point for a study of a segment of bacterial phylogeny and gene flow that may through application of appropriate experimental design ultimately establish criteria for better understanding of relationships within the entire order. The demonstration of nuclear interchange in several species of bacteria as well as indirect evidence of its occurrence in the genus Bacillus (Delamater, 1953; Johnson, 1960) support this theoretical approach.

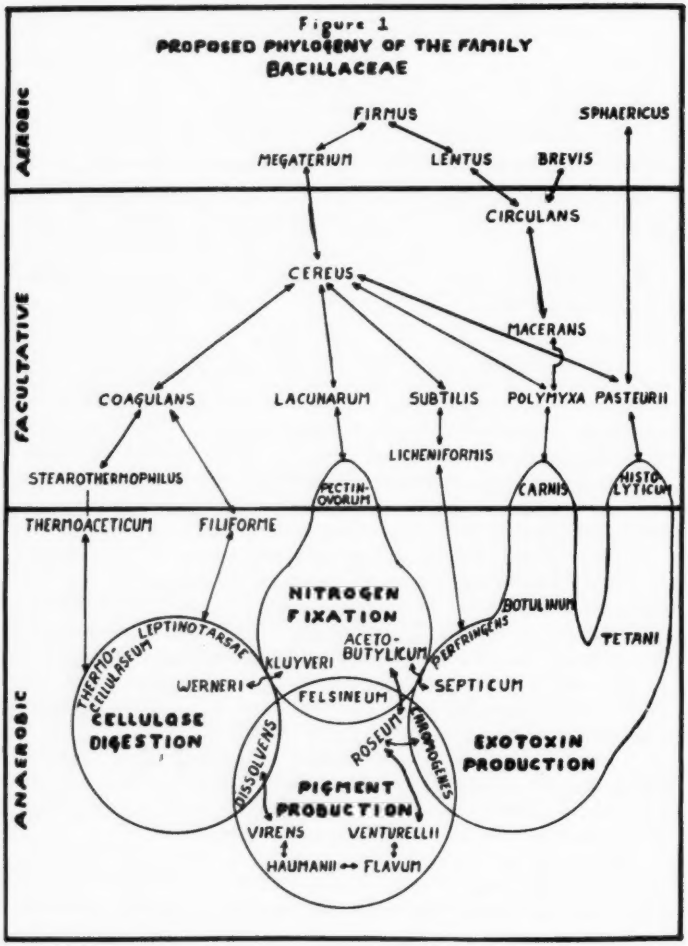
In reviewing the taxonomy of this family (Breed et al., 1957), it is apparent that nitrogen fixation, cellulose digestion and exotoxin production are not combined in any known species of the genus Clostridium. While most species in these three groups are anaerobic and non-pigmented, each possesses at least one species that is pigmented, and two of the three have at least one that is a facultative aerobe, suggesting a relationship in these two directions.

Since strict aerobes and strict anaerobes are logically and probably ecologically incapable of direct nuclear transfer, facultative members of both Bacillus and Clostridium were checked for intermediary characteristics.

Sub-criteria used in this analysis were: acid and gas from carbohydrates, acid only, no acid or gas from carbohydrates (without reference to specific sugars), gelatin liquefaction, acetyl-methyl-carbinol production, swollen or non-swollen sporangia, Gram stain reaction and optimum temperature.

Figure 1 shows the postulated relationship between species of the family, using key species for intermediates in the larger groups.

Figure 1
PROPOSED PHYLOGENY OF THE FAMILY BACILLACEAE



If strict anaerobes are considered primitive, the pigmented Clostridia seem to include the necessary characteristics for giving rise to three distinct groups of species, i.e. cellulose digesters, nitrogen fixers and exotoxin producers. The major characteristics of this pigmented group are: swollen sporangia, acid and gas from sugars, gelatin not liquified, temperature optima, Gram reaction negative to variable, and mesophilic.

The cellulose digesters are primarily: Gram-negative with swollen sporangia, gelatin negative and attack carbohydrates weakly or not at all.

The nitrogen fixers are primarily: Gram-positive with swollen sporangia, attack sugars with production of acid and gas, gelatin negative and mesophilic.

The exotoxin producers are primarily: Gram-positive with swollen sporangia, produce acid and gas from sugars, liquefy gelatin and are mesophilic.

Six distinct lines of development can be shown from these three major groups.

1. From the thermophilic cellulose-digesters through facultative aerobic thermophiles to the facultative thermophile Bacillus coagulans.
2. From the cellulose-digesters with non-swollen sporangia to B. coagulans.
3. From the facultatively aerobic nitrogen-fixers through Clostridium lacunarum to B. cereus.
4. From the exotoxin-producers with non-swollen sporangia through the antibiotic producers B. licheniformis and B. subtilis to B. cereus.
5. From the facultatively aerobic exotoxin-producers attacking sugars with acid and gas formation through B. polymyxa to B. cereus and B. circulans.
6. From the facultatively aerobic exotoxin-producers not attacking sugars through B. pasteurii to both B. cereus and B. sphaericus.

The work of Smith et al. (1946) has done much to clarify the interrelationships among the species of Bacillus. Their work indicated that B. coagulans, B. cereus and B. circulans are species with variable characteristics as might well be expected of species playing the intermediate role here assigned them.

The strict aerobes (genus Bacillus) in the family have in common inability to produce acetyl-methyl-carbinol, liquefy

gelatin (except B. lentus) and fail to attack sugars (except B. megaterium - acid, no gas). Taxonomic intermediates between B. cereus and B. megaterium are well known as well as the close relationship of B. pasteurii and B. sphaericus. B. circulans is viewed as giving rise to both B. lentus and B. brevis.

This analysis suggests that Gram-negativeness, pigment production, spores in swollen sporangia, non-liquefaction of gelatin, anaerobiosis, acetyl-methyl-carbinol production and acid and gas from sugar may be primitive characteristics of this family. Clostridium venturelli has these characteristics (VP reaction not reported). The converse of these reactions would represent the most advanced species (B. firmis).

That cellulose digestion, nitrogen fixation and exotoxin production occupy similar or identical gene loci is suggested. Except for the relationship of strict aerobiosis to lack of acetyl-methyl-carbinol production, all characteristics used here appear to operate independently.

The author realizes that modern phylogeny envisions a reticulate rather than mono-linear form of development. However, the present status of bacterial phylogeny suggests beginning with the latter in hopes that the former may be evolved to provide the field with a more definable concept of bacterial species.

Other criteria reported in the literature (Bradley and Franklin, 1958; Salton, 1956, Judith and Weinberg, 1959) have been used and are in general agreement with the scheme here given. However, the species covered by these authors have been too few to employ them as general criteria for this study.

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BOOK REVIEW

Bacteriological Nomenclature

Atlas of Bacterial Flagellation. Einar Leifson. January 8, 1960. 171 pp. Academic Press, Inc., New York.

The results of long study by Dr. Leifson of bacterial flagella and of the taxonomic significance of their distribution on the bacterial cell surface are summarized and significantly illustrated in this "Atlas". The author has been most active in the development of better techniques for observation of flagella based largely upon improved staining methods.

His photographs of stained preparations of representatives of some 56 genera of bacteria indicate clearly that for taxonomic studies recourse to higher magnifications such as those of the electron microscope are unnecessary. Photographs of three species from bacterial genera (Lachnospira, Lophomonas, and Succinovibrio) not listed in Bergey's Manual of Determinative Bacteriology are given.

The Leifson Atlas should be of material assistance in the better understanding of rational taxonomy of the bacteria. Correlation of morphology and physiology as in the acetic bacteria is emphasized. A study of the text and of the photographs shows clearly that flagellar distribution is a valuable criterion in bacterial taxonomy but that there are some real problems in its adequate use; for example, some of the typically polar flagellete organisms have additional non-polar flagella of a different wave length. In some genera extreme diversity of flagella distribution is evident; for example, in Listeria, different strains show wide differences in morphology of flagella, in some the flagella are "normal", some show small amplitude, some are straight and some are coiled.

Unfortunately, no attempt has been made in the Atlas to correlate the findings with type strains of species or type species of genera. Much additional work is needed to fit the advanced thinking of Leifson as given in the Atlas into an adequate taxonomic presentation of the bacteria.

The Atlas of Bacterial Flagellation should be available not only in laboratories in advanced courses in bacteriology, but should be available also in those of elementary students.

We may hope that these studies may be extended to type species and type strains, and integrated more fully into the type concept of modern biology.

R.E. Buchanan

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BREVIBACTERIUM LEUCINOPHAGUM SPEC. NOV.

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The genus Brevibacterium Breed was established in 1953 (1), and together with the genus Kurthia Trevisan constitutes the family Brevibacteriaceae Breed (2). The members of Brevibacterium are typically short, unbranched, gram positive, non-sporulating, rod-shaped bacteria. The type species is B. linens (Weigmann) Breed.

During a series of investigations of amino acid metabolism we have isolated from soil a new species of bacterium which possesses characters typical of the genus Brevibacterium. We propose the name Brevibacterium leucinophagum for this species.

A type culture has been deposited with the American Type Culture Collection, Washington, D. C. (ATCC No. 13809)

METHODS

The organism was isolated from a sample of flower bed soil. A few milligrams of soil were introduced directly into a medium containing 1% (w/v) L-leucine and mineral salts. After 24 hrs at 37° the heavily turbid broth was streaked on Petri plates containing the same medium solidified with 2% agar. Well isolated colonies were picked and maintained on plain nutrient agar slants.

Standard bacteriological techniques were used in the diagnosis of the species (3). Each test was repeated at least three or four times at intervals of approximately two months. Determination of fermentable carbohydrates was made by inoculation of tubes of plain nutrient broth supplemented with 0.5-1.0% of the carbohydrate and containing bromthymol blue and a Durham vial.

RESULTS

Cultural characteristics

Flasks of liquid media show silkiness when swirled after 8-12 hrs at 30°. Turbidity becomes moderately heavy, and

a thin membrane forms on the surface. This is easily dislodged and settles to the bottom while the broth remains turbid. The slight ring which remains soon gives rise to another membrane.

Colonies on the surface of gelatin or nutrient agar plates are small (1-2 mm in diameter), circular with entire edge, raised, butyrous and glistening. Colonies appear white to buff-colored when viewed by reflected light, iridescent by transmitted light. Agar streaks show moderate, beaded growth.

Gelatin and agar stabs show no growth, either in the presence or absence of fermentable carbohydrate. Similarly, glucose broth tubes overlaid with sterile mineral oil do not support growth.

Morphological characteristics

The cells stain readily and uniformly with the usual aniline dyes and are gram positive during the early phases of growth, but lose their gram positive character after 12-16 hrs at 30°. The cells are very short, stubby rods, 0.4-0.7 x 0.7-1.4 microns; young cells from liquid cultures often resemble cocci. At temperatures above 30° long, unbranched filaments and pleomorphic forms often occur. No characteristic cell arrangements are noted. Endospores are not formed. No lipid inclusion bodies are formed in carbohydrate media. The species is not motile.

Physiological characteristics

Acid, but no gas, is readily produced in glucose broth; methyl red tests are negative, however, and no acetylmethylcarbinol is formed. No acid or gas production was noted when fructose, lactose, sucrose, maltose, mannose, raffinose, glycerol, mannitol, sorbitol and salicin were tested. When first isolated, the species produced slight acidity but no gas in the presence of xylose, but this property was lost after several transfers on nutrient agar. A slightly basic reaction was usually noted in a peptone medium (pH 7.5-7.6 by the glass electrode), but no ammonia could be detected with Nessler's reagent.

Nitrates are not attacked, nor is urea. Good growth is obtained in 24-48 hrs at 30° when washed cells are inoculated into media containing ammonium phosphate as sole nitrogen source.

Since B. leucinophagum does not grow anaerobically in stab cultures, gelatin hydrolysis was tested by the Frazier method as modified by Smith (4). Gelatin is not hydrolyzed. Hydrogen sulfide production was not detected when agar plates containing either lead acetate or ferrous sulfate were inoculated with the test organism. Starch is not hydrolyzed.

Indole is not produced in Bacto-tryptone (Difco) broth cultures. Repeated subculture in the synthetic medium of Koser yields heavy growth, indicating that citrate is readily utilized as sole carbon source. Other intermediates in the tricarboxylic acid cycle (α-ketoglutarate, succinate, malate, fumarate and acetate) are rapidly utilized, suggesting operation of this cycle.

When tubes of litmus milk were incubated at 30°, no immediate changes took place. After 4-5 days, however, the litmus in the depths of the tubes was slowly reduced, and after 10-12 days the contents of the tubes became solid. Litmus at the surface remained blue, indicating that the curd which formed was not due to accumulation of acid. No peptonization of the casein was apparent, however, even after 14 days.

No hemolysis occurs on sheep blood agar plates at 30° or 37°.

Cultures of this species remain viable for periods up to 3 months when agar slants are stored at 4°; broth cultures at this temperature do not remain viable longer than 3-4 weeks, however. Cultures grow well, though slowly, at 10°, and show optimum growth at 30°. At 37° many long, unbranched filaments and pleomorphic forms are formed rapidly in liquid media. No growth occurs at 45°.

Manometric experiments with washed, whole cells showed very high rates of oxygen uptake on L-leucine (700-800 microliters per hr per 150 mg wet cells). The mechanism of dissimilation of leucine by this species will be reported elsewhere.

DISCUSSION

The properties of this species indicate that it is properly classified in the family Brevibacteriaceae Breed according to the 7th edition of Bergey's Manual. This family includes two genera, Brevibacterium and Kurthia, which are differentiated primarily by the fact that members of the latter

genus form long, unbranched rods or filaments and do not utilize carbohydrates. Although long, unbranched rods have been observed in cultures of the organism described here, this occurs only at temperatures above the optimum, and has never been observed at lower temperatures. In addition these filamentous forms are accompanied by the presence of distorted, swollen and club-shaped cells which are typical of the pleomorphic forms found in bacterial cultures exposed to degenerative influences. Serial microscopic examination of developing cultures shows no evidence that these filaments are formed first and then divide into coccoid elements, as is described for the genus Kurthia.

Although 23 species of Brevibacterium are listed in the 7th edition of Bergey's Manual, a careful comparison of cultural, morphological and physiological characteristics shows that the organism described here is not identical to any of these. This species is non-motile; therefore it is not B. incertum, B. imperiale, B. lipolyticum, B. acetylicum, B. sulfureum or B. helvolum. It produces no pigments when grown on agar, and is therefore not B. linens, B. erythrogenes, B. fulvum, B. insectiphilium, B. brunneum, B. vitarumen, B. maris or B. fuscum. Other properties differ sufficiently also to show that it is not a pigmentless variety of these species. Nitrites are not produced from nitrates; therefore this species is not B. stationis, B. quale or B. ammoniagenes. B. leucinophagum does not liquefy gelatin, as do B. sociovivum, B. immotum and B. marinopiscosum. Furthermore, sea water or similar saline solutions are not required to initiate growth. The organism described here is not identical to B. minutiferula since the latter produces slight acidity from sucrose and in litmus milk. B. leucinophagum possesses none of the cultural characteristics of B. healii. Finally, it produces acid from glucose only, whereas B. tegumenticola produces acid from glucose, maltose and sucrose.

The evidence thus warrants the proposal of a new species.

SUMMARY

A bacterium, isolated from soil with L-leucine as sole carbon and nitrogen source, was found to possess properties typical of the genus Brevibacterium Breed, family Brevibacteriaceae Breed. It is a new species for which the name Brevibacterium leucinophagum is proposed.

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INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
Volume 10 No. 3 July 15, 1960 p. 219

J.C. BROOM, M.D.
1902 -- 1960

An Appreciation

On 16th March Dr. J.C. Broom died suddenly at his home in London after a short illness. Born in Scotland he studied medicine at the University of St. Andrews, qualified in 1924 and took his degree of M.D. in 1929. A year before he had joined the Wellcome Bureau of Scientific Research (now the Wellcome Laboratories of Tropical Medicine) in London and has since remained there.

In his early bacteriological work he collaborated with Dr. H. C. Brown in devising the well-known "Brown's Opacity Tubes", used extensively in the standardization of vaccines. For his work during the last war, when he was in charge of the Wellcome Yellow Fever Immunization Centre, he was awarded the Order of the British Empire.

John Broom was perhaps best known for his work in the field of leptospirosis after the war. He was a founder member and Secretary of the Leptospira Subcommittee of the International Committee on Bacteriological Nomenclature and through his active work and personal qualities became one of the foremost authorities in this branch of science and a dear friend to all members of the Committee who had the privilege to meet him on several occasions, when his sound advice was held in high esteem.

He was the author and co-author of more than 40 scientific papers and studies, of which his book "Leptospirosis in Man and Animals" (Livingston Ltd. 1958), published in association with Dr. J.M. Alston, has already become a classic in its field.

The remembrance of his charming personal qualities will be cherished by all who came in contact with him.

J.W. Wolff, M.D.
Chairman of the Leptospira
Subcommittee

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
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NEWS AND NOTES

The following notice has been received from the Department of Scientific and Industrial Research, Charles House, 5-11 Regent Street, London, S.W. 1, England.

NATIONAL COLLECTION OF INDUSTRIAL BACTERIA

The National Collection of Industrial Bacteria has been transferred from the National Chemical Laboratory to the Torry Research Station, D.S.I.R. All future inquiries and correspondence concerning the Collection should be addressed to The Curator, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen.

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
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THE DIFFERENTIATION OF AEROMONAS AND C27 CULTURES FROM ENTEROBACTERIACEAE

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The reactions given by cultures of Aeromonas in the biochemical tests commonly used in the diagnostic laboratory often resemble those of Enterobacteriaceae and the differentiation of Aeromonas may be difficult. Frequently, however, strains of Aeromonas can be identified accurately if a number of media are employed and if the reactions obtained are compared with those given by members of the various groups of Enterobacteriaceae. Also, flagellar stains are of assistance in this differentiation.

In a search for a more rapid, as well as accurate, method of distinguishing Aeromonas cultures, we tried the cytochrome oxidase test employed by Gaby and Hadley (1957) for the identification of Pseudomonas aeruginosa. The test media employed by these investigators were nutrient broth tubed in 4.5 ml amounts and nutrient agar or blood agar plates. Cultures to be tested were incubated for 18 to 24 hours at 37°C or at room temperature (24 to 28°C), depending upon which was optimal. Following incubation, 0.2 ml of a 1.0 per cent solution of alpha-naphthol in 95 per cent ethyl alcohol and 0.3 ml of a 1.0 per cent aqueous solution of para-aminodimethylaniline oxalate were added to each broth culture. The cultures were then shaken. The appearance of a blue color was indicative of the presence of cytochrome oxidase. Plate cultures were tested by allowing a few drops of an equal-parts mixture of the two reagents to flow over isolated colonies.

The authors first employed the method as described above but subsequent tests indicated that nutient broth tubed in 1.0 ml amounts gave results comparable to those obtained with larger volumes and long nutrient agar slants yielded superior results. Hence, nutrient agar slant cultures, incubated at 37°C, or at a lower temperature if required, for 18 to 20 hours were employed in the present studies. After

incubation, two or three drops of each reagent were introduced and the tubes were tilted so that the reagents were mixed and flowed over the growth on the slants. Positive results were indicated by the development of an intense blue color in the growth within 30 seconds. Occasional cultures required up to one minute to give intense reactions. Rare strains required up to two minutes and produced less intense or spotty reactions. Any doubtful or very weak reaction that occurred after two minutes was ignored.

The results of cytochrome oxidase tests with cultures representative of the various groups of Enterobacteriaceae, with Aeromonas, and Aeromonas-like strains, as well as with members of several other bacterial groups, are given in Table 1. While none of the Enterobacteriaceae tested gave even a weakly positive reaction, all of the strains of Aeromonas, Aeromonas-like organisms (C27), Vibrio, and Pseudomonas reacted rapidly with the production of an intense blue color. Although only a very few cultures of Alcaligenes were tested, all were positive within 30 seconds. Conversely, the small number of strains of Xanthomonas tested were negative. After most of the work reported was completed, the authors came upon the work of Griffin et al. (1952, 1953) in which it was reported that cultures of Aeromonas salmonicida were oxidase positive. However, this was the only reference found to the use of the test with Aeromonas and we found no reference to its use with Vibrio comma or other vibrios.

The biochemical and serological reactions of 40 Aeromonas and 48 C27 (v. inf.) strains were studied in detail and the results will be reported elsewhere. However, tests that proved to be of particular value in the differentiation of strains of Aeromonas and of Aeromonas-like cultures and those of the Enterobacteriaceae with which they are more likely to be confused, are summarized in Tables 2 and 3. Attention is directed to the value of the decarboxylase tests in effecting this differentiation. The majority of the Aeromonas cultures were negative in lysine medium, strongly positive in arginine, and negative in ornithine medium. This pattern of reaction was particularly valuable in the differentiation of Aeromonas and the Enterobacteriaceae. Among the latter, only Citrobacter strains reacted in a similar manner and in that instance the reactions in arginine medium were delayed 2 or 3 days and were less intense

Table 1. Results of Cytochrome Oxidase Tests

	No. Tested	No. +	No. -
Enterobacteriaceae (1222 cultures)			
Escherichia	222	0	222
Shigella	152	0	152
Salmonella	102	0	102
Arizona	61	0	61
Citrobacter	108	0	108
Klebsiella	54	0	54
Aerobacter	144	0	144
Hafnia	56	0	56
Serratia	79	0	79
Proteus	134	0	134
Providence	110	0	110
Aeromonas, *Vibrio*, etc. (279 cultures)			
Aeromonas species	36	36	0
C27	48	48	0
Vibrio comma	115	115	0
Vibrio species	12	12	0
Pseudomonas species	15	15	0
Xanthomonas species	4	0	4
Alcaligenes species	5	5	0
Flavobacterium species	10	2*	8
Erwinia species	34	3*	31

*These reactions were weak and slow.

than those given by *Aeromonas* (Ewing, Davis, and Edwards, in press).

The differential reactions of 48 strains of an *Aeromonas*-like biotype (C27) are summarized in Table 3, along with those given by *Aeromonas formicans*, *Aeromonas salmonicida*, and some *Enterobacteriaceae* for which they may be

Table 2. Differentiation of *Aeromonas* (Based on the Reactions given by the Majority of Cultures)

	Serratia	*Aerobacter* C*	*Hafnia*	*Klebsiella oxytoca*	*Aeromonas***
Gas from glucose	d	+	+	+	d
Arabinose	-	+	+	+	+ or -
Inositol	+ or (+)	+	-	+	-
Rhamnose	-	-	+	+	-
Xylose	d	+	+	+	-
Raffinose	-	+	-		-
Sorbitol	+	+	-	+	-
Dulcitol	-	-	-	d	-
Adonitol	+ or (+)	-	-	+	-
Indol	-	-	-	+	+ or -
MR 37C	-	d	+	-	+
22C	-	-	-	-	d
VP 37C	+	d	d	+	d
22C	+	+	+	+	d
Lysine decarboxylase	+	+ or (+)	+	+	-
Arginine dihydrolase	-	-	-	-	+
Ornithine decarboxylase	+	+	+	-	-
Gelatin	+	+	-	+	+ or (+)
Cytochrome oxidase test	-	-	-	-	+

+ positive in 1 or 2 days.
(+) delayed positive.
+ or - majority of strains positive, negative varieties occur.
d different biochemical types (+, (+), or -).
* *Aerobacter* subgroup C corresponds to *A. liquefaciens* (Grimes).
** Forty cultures, including *A. formicans*, *A. salmonicida*, and other described species.

Table 3. Differentiation of Aeromonas, C27, etc.

	A. salmonicida	A. formicans	C27	Shigella sonnei	E. coli 0112a, c	E. coli
Gas from glucose	-	-	-	-	-	+ (-)
Mannitol	+	+	-	+	-	+
Dulcitol	-	-	-	-	(+)	d
Rhamnose	-	-	-	+ or (+)	+	+
Arabinose	(+)	+	-	+	+	+
Inositol	-	-	+	-	-	-
Xylose	-	-	-	-	-	+
Raffinose	-	-	-	+ or (+)	+	d
Sorbitol	-	-	-	-	+	+
Salicin	+ or (+)*	+	(+) or -	-	d	d
Indol	-	+ or -	+	-	+	+
Simmons' citrate	-	+ or (+)	-	-	-	-
Gelatin	+	+[3]	-	-	-	-
KCN	-	+	-	-	-	-
Mucate	-	-	-	-	+	+
Lysine decarboxylase	-	-	+	-	-	d
Arginine dihydrolase	-	+	+	- or (+)	- or (+)	d
Ornithine decarboxylase	-	-	d	+	-	d
Cytochrome oxidase test	+	+	+	-	-	-

*Gas formed slowly from salicin.

mistaken. The resemblance of the C27 strains to Shigella sonnei was superficial but the reactions of the latter were included for comparative purposes. Seventy strains of the C27 biotype were collected and studied at intervals during the past 16 years by the senior author. Of these 48 were available for the work reported here as well as for more detailed studies to be reported elsewhere. Ferguson and Henderson (1947) apparently gave the first description of this microorganism, to which they applied the designation C27. These investigatiors reported that the flagellation of the microorganism was amphitrichous. Further, they demonstrated that the O antigens of the C27 strain were identical with those of S. sonnei form I, a fact that has been confirmed by several investigators. Also, Bader (1954) described a bacterium, the O antigens of which were related to those of S. sonnei and assigned the microorganism to the genus Pseudomonas on the basis of its polar monotrichous flagellation. The authors procured this strain (M51) from Dr. Bader in 1955 and demonstrated that its O antigens were identical with those of the original C27 culture of Ferguson and Henderson. Twenty of the above-mentioned 48 C27 strains were antigenically related to S. sonnei, the remainder were not.

In spite of these reports on the polar flagellation of C27 strains, some workers have more or less tacitly accepted them as Enterobacteriaceae, although they have never been assigned to any particular group in that family. As a result of the studies herein reported the authors were convinced that the C27 bacteria do not belong in the family Enterobacteriaceae and that their resemblance to certain members of that family was only superficial. All of the cultures utilized glucose fermentatively in the medium of Hugh and Leifson (1953), and hence should not be placed in the genus Pseudomonas. They differed from described species of Aeromonas in some respects but appeared to be more closely related to members of that group than to any other. Further, studies on the flagellation of C27 strains, kindly made by Dr. Rudolph Hugh of George Washington University, have confirmed the fact that the flagellation of the cultures was predominately polar monotrichous and that the morphology and variation of the flagella was like that of Aeromonas.

Summary

The results of the studies reported indicate that the cytochrome oxidase test affords a rapid, convenient, and accurate method for the differentiation of Aeromonas and Aeromonas-like cultures from members of the family Enterobacteriaceae and that the differentiation thus effected can be confirmed by the pattern of reaction obtained in other biochemical tests. Further, the investigations indicate that the differentiation of certain important vibrios, such as Vibrio comma, from the Enterobacteriaceae can be effected in a similar manner.

Addendum

After this manuscript was prepared, the authors received a reprint of an article entitled Studies on So-called Paracolon C27 (Ferguson) by Sakazaki, R., S. Namioka, R. Nakaya, and H. Fukumi (Japan. J. Med. Sci. Biol. 12: 355-363) in which it was concluded that the C27 bacteria should be classified in the genus Pseudomonas, and in which the name Pseudomonas michigani was proposed. The authors cannot agree that the "C27" bacteria should be so classified, since the authors' work has shown that among other things they are fermentative forms, and probably should be placed in the genus Aeromonas. Further, the specific epithet "shigelloides" (Pseudomonas shigelloides, Bader 1954) may have priority. These matters will be discussed in detail in a forthcoming publication.

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